

Polyphosphazene polymers: The next generation of biomaterials for regenerative engineering and therapeutic drug delivery

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ABSTRACT

The demand for new biomaterials in several biomedical applications, such as regenerative engineering and drug delivery, has increased over the past two decades due to emerging technological advances in biomedicine. Degradable polymeric biomaterials continue to play a significant role as scaffolding materials and drug devices. Polyphosphazene platform is a subject of broad interest, as it presents an avenue for attaining versatile polymeric materials with excellent structure and property tunability, and high functional diversity. Macromolecular substitution enables the facile attachment of different organic groups and drug molecules to the polyphosphazene backbone for the development of a broad class of materials. These materials are more biocompatible than traditional biomaterials, mixable with other clinically relevant polymers to obtain new materials and exhibit unique erosion with near-neutral degradation products. Hence, polyphosphazene represents the next generation of biomaterials. In this review, the authors systematically discuss the synthetic design, structure-property relationships, and the promising potentials of polyphosphazenes in regenerative engineering and drug delivery.

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I. INTRODUCTION

Advances in regenerative engineering and therapeutic drug delivery have sparked a new age in the design of new advanced biomaterials with a broad spectrum of physical, chemical, and biological properties.^{1–4} The inherent physicochemical properties, biodegradability, and biocompatibility of biomaterials are the deciding factors for the success in their utilization in regenerative engineering.⁴ Despite the advances made so far, there are numerous unaddressed issues in the biomedical applications of biomaterials, and these issues are related to the inability of biomaterials to present appropriate cues

for favorable interactions between the materials and the cells for specific regenerative purpose.^{5–9} Therefore, the need exists for the development of a broader range of synthetic polymers to meet the ever-changing demands and complex requirements of different medical applications where currently used polymers are deficient.

The exceptional design flexibility of polyphosphazenes polymers provides a versatile platform for the development of tunable polymers that can be used alone or in combination with other clinically relevant polymers to meet application-specific needs and requirements.^{10–12} The physiologically benign and neutral pH of

polyphosphazenes' degradation products has distinguished them as an exciting and unique class of biomaterials when compared to widely used polyesters.^{2,13}

This class of polymers is based on the inorganic backbone with alternating nitrogen and phosphorous atoms, and each phosphorous atom bears two substituents, with different types of groups available for the optimization of the material properties.^{1,14} Polyphosphazene-based biomaterials have shown great promise in their use in the field of biomedicine, and their high prospects as matrices for tissue regeneration have been demonstrated in several studies as homopolymers, random and block copolymers, polymer blends, and composites.^{1,14–18} For medical uses such as tissue regeneration and drug delivery, the substituent side groups are hydrolytically sensitive and biocompatible.¹⁹ The rationale for using side groups that promote hydrolytic sensitivity stems from the fact that they have the ability to sensitize the polyphosphazene backbones to hydrolysis, which allow them to break down into nontoxic small molecules that are resorbable.^{12,20}

The most common class of these medical polyphosphazenes is those with amino acid and peptide esters as side groups. The hydrolytic vulnerability of the amino acids and peptide ester substituents allows the permeation of water molecules to the polyphosphazenes backbone, causing it to break down into ammonia, phosphates, and corresponding side groups.^{1,14,20} The resultant degradation products have a buffering effect as ammonium phosphate is naturally an amphoteric compound.^{19,21} An amphoteric compound exhibits acidic or basic tendencies depending on the pH of their environments.²¹ This neutralizing behavior of polyphosphazene degradation products is the main focus of interest in the fabrication of polyphosphazene blends with FDA-approved polyesters such as poly(lactic-co-glycolic acid) (PLGA), polylactic acid, polyglycolic acid, and polycaprolactone (PCL).^{21–23} The hydrolysis products of polyphosphazenes are capable of stabilizing the pH of their microenvironments and, in other words, can neutralize the acidic degradation products of the polyesters in a polyphosphazene-polyester blend system.^{22–24}

This review gives an overview on the use of polyphosphazenes in biomedical applications, mainly regenerative engineering and drug delivery. For regenerative engineering, in as much as tremendous progress has been made using other degradable polymeric materials, there remain considerable challenges in terms of toxicity and degradability as there are no one-size-fits-all biomaterials, and polyphosphazenes are promising in this regard. Concerning drug delivery, current therapeutics is characterized by limitations such as nonspecificity in distribution leading to overdose, poor pharmacokinetics, and significant side effects. Due to these issues, there is an increasing demand for a drug delivery system that can deliver drugs to a specific target without having detrimental effects on other sites.

II. SYNTHESIS AND PREPARATION

Different synthetic routes have been developed for the synthesis of polyphosphazenes but the two-step reaction process, namely, ring-opening polymerization (ROP) followed by macromolecular substitution reaction, is most commonly employed for the design of most bioerodible species (Fig. 1).^{25–27} A cyclic inorganic

compound, hexachlorocyclotriphosphazene (HCCTP), is the starting material for most of the polymerization and is produced commercially using phosphorus pentachloride and ammonium chloride.²⁶ The synthesis of polydichlorophosphazene (PDCP) is the first step, and it is attained through the controlled thermal ROP of the HCCTP at 250 °C under high vacuum.^{1,28–30} Macromolecular substitution reactions constitute the second step, and it is achieved by the selective substitution of chlorine atoms on the PDCP with the desired nucleophile or a combination of nucleophiles.^{25,31} PDCP is a prepolymer which represents an intermediate stage in the polymerization process, and it is unstable and highly sensitive to air and moisture.²¹ The high reactivity is due to the labile nature of P-Cl, which can be manipulated through the unique macromolecular substitution of chlorine atoms with organic nucleophiles to yield stable polymeric species with unprecedented structural diversity.^{10,13,32} The nucleophilic side groups are either incorporated into phosphorous through nitrogen or oxygen. The effects of these linkages will be discussed in Sec. II D.^{15,33,34}

A. Ring-opening polymerization

Although other techniques exist, ROP remains the best-known process of obtaining the reactive intermediate, PDCP.^{14,20} This is achieved by carefully subjecting HCCTP to a high temperature of 250 °C in a sealed tube under vacuum. This technique usually results in a linear, high molecular weight PDCP with a broad molecular weight distribution (Fig. 1).^{26,35} The cleavage of the chlorine moieties occurs at a temperature around 250 °C, where there is a formation of cationic phosphazonium intermediates and ultimate initiation/propagation of the polymerization.³⁶ Temperature conditions can play a critical role in the degree of polymerization as significant crosslinking may occur with higher temperatures.^{1,27,37} However, at a temperature lower than 250 °C, the polymerization rate is low and thus leading to a minimal cleavage of chlorine moieties.¹⁴ Temperature-induced crosslinking hampers the dissolution of the PDCP prepolymer in organic solvents and prevents its further functionalization.^{1,19,22,26,38,39} Moreover, the use of a Lewis acid, such as anhydrous AlCl₃, as a catalyst allows the use of a lower polymerization temperature (around 200 °C), and this consequently leads to an enhanced yield.^{4,23,25} Also, ROP can be carried out in a more facile solution state route, which utilizes solvents such as trichlorobenzene, at a temperature of 214 °C with CaSO₄·2H₂O and HSO₃(NH₂) as cocatalysts.²⁵ The solution state technique enhances conversion and yield of ROP, resulting in PDCP with a relatively narrow and monomodal molecular weight distribution.²⁵ In as much as the ROP holds great importance, disadvantages exist for this strategy as it requires a high polymerization temperature to initiate the ring opening and it is characterized by uncontrollable molecular weight, and low monomer conversion.¹² Consequently, living cationic polymerization was devised to address these limitations.

B. Living cationic polymerization

This is an alternative synthetic procedure developed to produce polyphosphazene derivatives with uniform size distributions and relatively lower molecular weights.^{20,28,40} The strategy entails the condensation of monomeric trichloro(trimethylsilyl) phosphoranimine [(CH₃)₃Si-N≡PCl₃] catalyzed by phosphorus

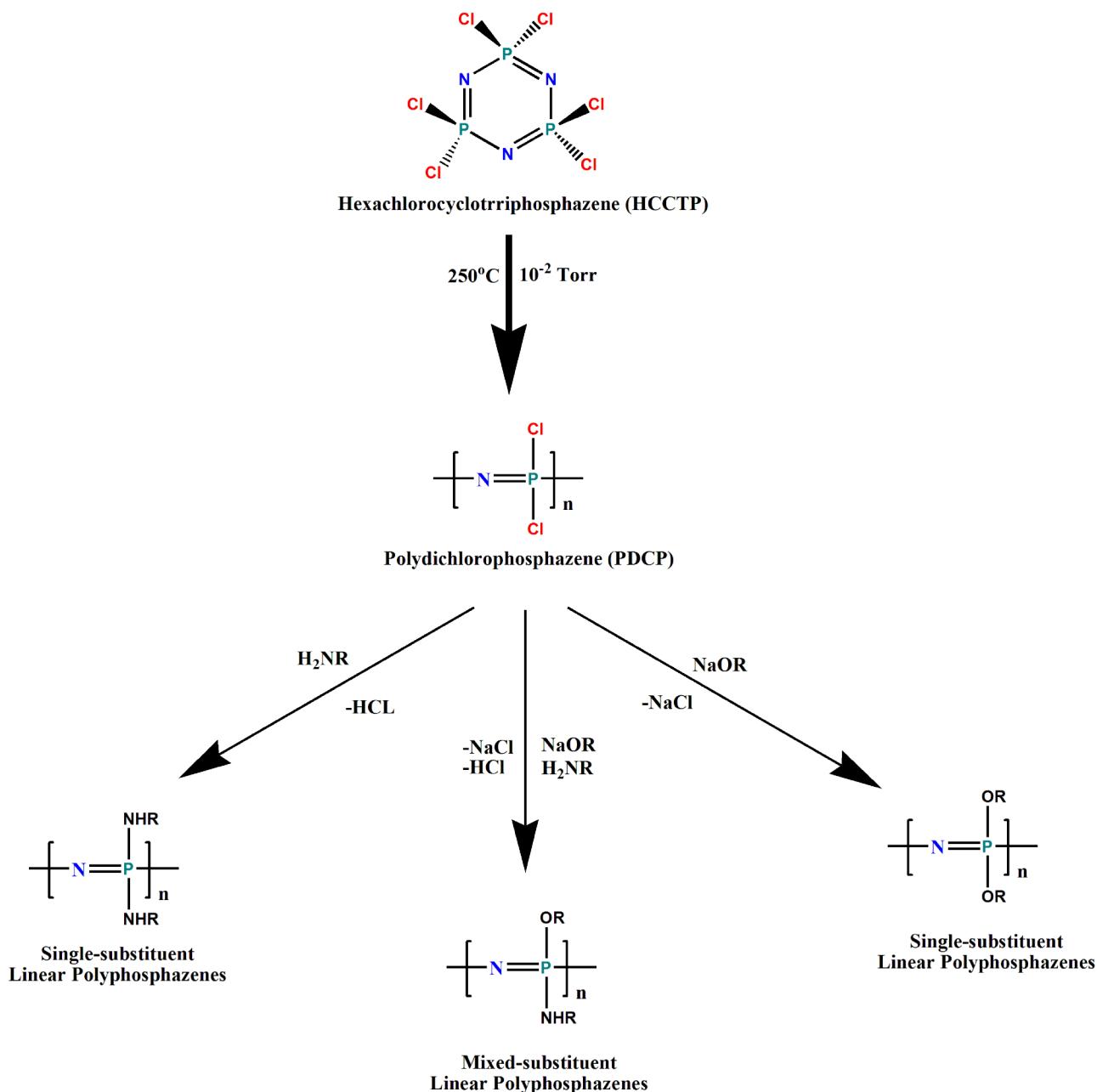


FIG. 1. Overall synthetic pathway to poly(organophosphazenes). The first step involves the ring-opening polymerization (ROP) of hexachlorocyclotriphosphazene (HCCTP) and the second step entails the nucleophilic macromolecular substitution of polydichlorophosphazene.

pentachloride (PCl_5) in chloroform at ambient temperature.¹ This polymerization is usually carried out in a solution state, where an initiation step ensues through the reaction of one monomer of $\text{Cl}_3\text{PNSiMe}_3$ with two equivalents of PCl_5 to yield transient cationic species $(\text{Cl}_3\text{PNPCl}_3)^+$ ⁴¹ with PCl_6^- as the counterion for stabilization.^{21,42} The intermediate species propagate the chain growth

by reacting with the $\text{Cl}_3\text{PNSiMe}_3$ monomers until they are completely consumed, leaving a polymer chain with living cationic end groups.²³ These living cationic end groups allow for the reactivation of the chain growth polymerization when monomeric $\text{Cl}_3\text{PNSiMe}_3$ are introduced. For every molecule of monomers added, one equivalent of ClSiMe_3 is generated as a by-product,

making it as a special case of polycondensation that are propelled by a chain growth mechanism.^{4,19,43} The presence of the living end groups aids in controlling and defining the compositions and size distributions of the polymer with the monomer to cationic initiator ratios influencing the chain size.^{16,26,37} Polymerization time and monomer concentration can be varied to modulate and obtain the desired polymer chain length.

C. Synthesis of poly (organo) phosphazenes by direct routes

In order to streamline the synthesis of polyphosphazenes, Steinke *et al.*⁴⁴ designed a more straightforward and efficient methodology that utilizes the single step that skipped the PDCP precursor in the direct preparation of poly (bistrifluoroethoxy phosphazene). This technique involves the anionic polymerization of *N*-silylphosphoranimines using H₂O as an initiator and *N*-methylimidazole as a catalyst at an accessible temperature of 125 °C. The poly (bistrifluoroethoxy phosphazene) polymer exhibited a tunable molecular weight with respect to monomer and initiator contents, high conversion (75%–99%), low polydispersity, and living polymerization kinetics. The results of this study affirm this route as a simpler alternative, and if a broader range of monomers could be explored and optimized, the direct synthesis technique creates an avenue to develop polyphosphazenes with efficient control and tunability over their molecular weight and polydispersity.⁴⁴

D. Macromolecular nucleophilic substitution

Macromolecular substitution is a postpolymerization step that allows the linkage of a variety of different side groups to the inorganic backbone of linear polyphosphazene through reactions that permit the regulation of the structures and properties of the final product by the electronic and steric features of the side groups (Fig. 1).^{26,28} The properties of the polymer products can vary from rubbery materials with glass transition down to -100 °C to brittle polymers with a glass transition temperature of 200 °C or higher.¹ The postfunctionalization involves the substitution of chlorine in the PDCP with organic or inorganic nucleophiles.^{10,30} The reactive P-Cl bonds are the impetus for these substitution reactions as it can either be hydrolyzed/crosslinked when exposed to moisture/oxygen or the chlorine atoms can easily be replaced with either oxygen-based nucleophiles (such as aryloxides and alkyl oxides) or nitrogen-based nucleophiles (such as primary amines).^{4,12} The resulting P-O and P-N bonds and the corresponding functionalized polyphosphazene are thermodynamically stable, as this helps in pushing the equilibrium to the right.^{19,43} Polyphosphazene polymers with P-O linkages exhibit more resistance to hydrolysis than the ones with P-N linkages and hence aryloxy-substituted and alkoxy-substituted polyphosphazenes are more hydrolytically stable than amino acid esters and dipeptide esters containing polymers in near-neutral media.¹ The substitution of the halogen atoms in PDCP is dependent on factors such as the nature of nucleophiles, reaction conditions, byproduct solubility, and solvent type. A bulky nucleophile would experience difficulty in replacing the chlorine atoms along the PDCP backbone due to high steric hindrance.^{1,25} A stronger nucleophile, such as fluoroalkoxides or fluoro- or nitro-

aryloxides, would be more aggressive in the attack for the substitution of the chlorine atoms.¹² In contrast, relatively weaker nucleophiles such as alkoxides or aryloxides are less aggressive in the substitution and, as such, require longer reaction time and higher reaction temperature to facilitate the substitution.^{26,29} The synthesis of mixed-substituent polyphosphazenes is feasible by employing two or more different nucleophiles, which can be substituted into the PDCP chains by either a simultaneous or sequential cosubstitution.¹⁵ The ratios of the substituents provide a platform that is not only used to fine-tune the properties of the polymer but to create specific physical interactions with other polymers during blending.^{20,28,37} This offers a broader design window for obtaining many more polymeric systems for numerous applications. Finally, to ensure a simple macromolecular substitution of new side groups, it is wise for researchers to utilize small molecule model reactions first for exploratory studies.²⁸ This kind of reaction involves the interactions of HCCTP and each of the intended organic side groups. The resulting products from the reaction can be characterized using ³¹P NMR and x-ray crystallography.^{12,22}

E. Cyclomatrixpolyphosphazenes

Cyclomatrixpolyphosphazenes (cyclo-PPHOS) is a class of highly cross-linked and monodispersed polyphosphazene nanoparticles, which are based on the one-pot polycondensation of HCCTP and a difunctional compound such as dichlorofluorescein (FD), 4,5-dibromofluorescein, 4,4 sulfonyldiphenol, dopamine, etc. [Fig. 2(a)].⁴⁵ As a result of steric hindrance, only one chlorine atom on each phosphorous atom of HCCTP can be substituted by the difunctional group.³³ Recently, there has been a rising interest in the use of cyclo-PPHOS for drug delivery and cell imaging applications.^{1,39,45–48} The typical synthesis of linear polyphosphazenes requires harsh reaction conditions (such as anhydrous and oxygen-free environment, and high pressure and temperature), which may drastically hamper its massive scale-up and commercialization.^{28,37} Besides, it is not very easy to control the properties and performance of a linear polyphosphazene-based product because of its high molecular weight (Mw > 10⁶ Da) and broad molecular weight distributions.¹² The high polydispersity is due to the nature of the ROP, which is characterized by uncontrollable initiation and propagation stages.¹⁵ The facile one-stage polymerization method through the precipitation polycondensation of HCCTP and difunctional moieties presents a springboard for scaling up the synthesis of polyphosphazene polymers as compared to the conventional ROP. The enhanced surface area provided by the resulting polymer spheres and the enormous hollow interior due to the arrangement of spheres makes them suitable for use in applications such as drug delivery vehicles [Figs. 2(b) and 2(c)].^{45,48,49} The morphology and size of the nanoparticles can be modulated by varying the type of solvent used during polymerization and the composition of the reactants.^{45,48,49} The solvent employed influences the reaction rate and morphological size of the HCCTP-based nanospheres because various solvents exhibit different polarity and solubility parameters.⁵⁰ The amount of HCCTP and the difunctional crosslinker is directly proportional to the size of the spheres as an increase in the starting materials yields relatively higher nanospheres' average diameter.^{45,48,49}

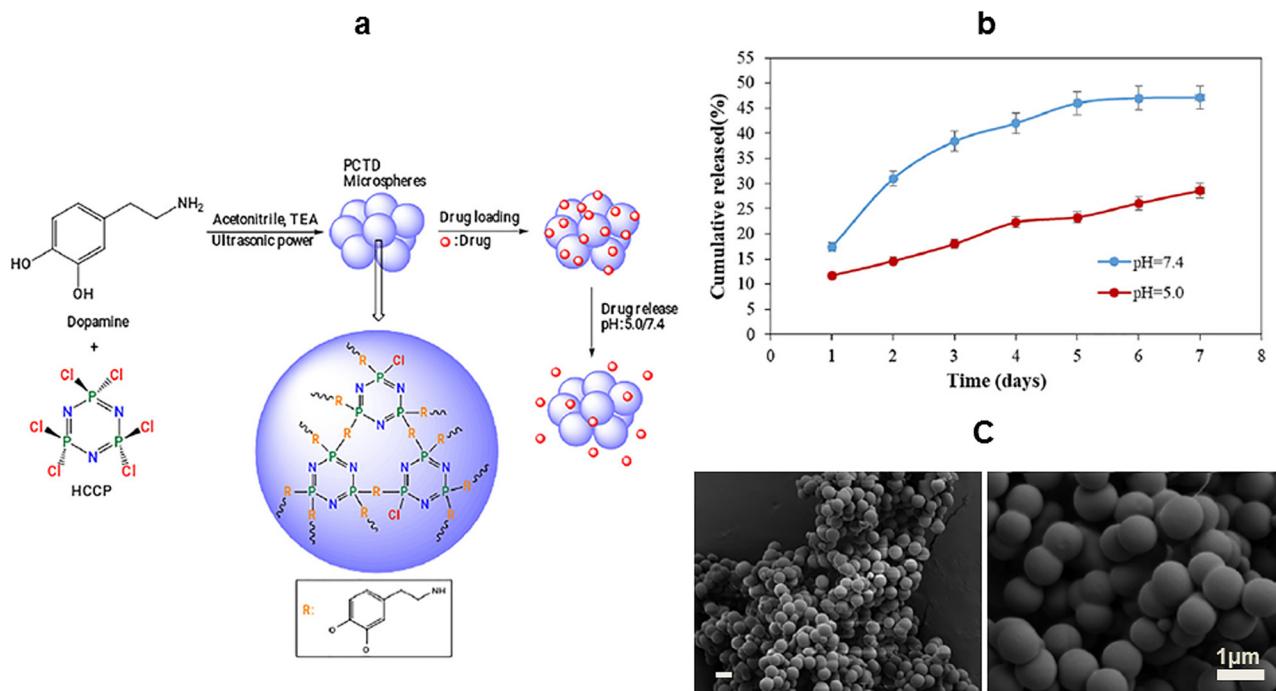


FIG. 2. Cyclomatrixpolyphosphazenes. (a) Preparation of cyclo-PPHOS microspheres and their drug loading and release. (b) *In vitro* study showing the release of acriflavine from cyclo-PPHOS microspheres in buffer systems. (c) SEM images of cyclo-PPHOS microspheres. Reprinted with permission from Metinoğlu Örüm and Süzen Demircioğlu J. Macromol. Sci. Part A Pure Appl. Chem. **56**, 9 (2019). Copyright 2019 Taylor & Francis.

III. CONTROL OF STRUCTURE-PROPERTY RELATIONSHIPS

The chemical characteristics of the backbone and side groups of any polymer can have a tremendous bearing on its overall properties.^{24,43} For the polyphosphazene system, the macromolecular substitution offers two useful ways to control and manipulate properties.²⁰ First, the polyphosphazene polymers are uniquely different from any other polymers as they are “inorganic” due to the elements (P and N) on the skeletal backbone but, at the same time are “organic” based on the chemistry and structures of the side groups.¹² The organic component is responsible for features such as thermo-oxidative stability, ligand coordinating activities, and mediating the side groups’ behaviors.^{1,29,37} In contrast, the side groups shield the backbone from hydrolytic attack or encourage the attack by allowing the ingress of water to the backbone, depending on the type of group.^{2,3,13} Also, the side groups direct the torsional mobility of the backbone and have a massive impact on how the polymer interacts with each other, other polymers, and its environments.^{2,3,51,52} As a whole, the additive effects of the inorganic skeletal backbone and the organic side groups give rise to the practical versatility of polyphosphazenes.

A. Influence of backbone

The peculiar traits of the phosphorous-nitrogen backbone are established on all the polyphosphazene-based polymers, and these

traits include flame retardancy, fire resistance, extreme chain flexibility caused by low barrier to torsion (0.1–0.5 kcal/repeating unit) of the skeletal bonds, and transparency through the visible to near-ultraviolet (220 μm) region of the spectrum.^{1,53} Besides, the light transmission exhibited by polyphosphazenes can depend on the side groups.¹ The backbone can also become susceptible to hydrolytic degradation to phosphate and ammonia when certain side groups are utilized.²⁰ This property is of valuable interest in the development of bioerodible biomaterials as nontoxic and neutral degradation products are highly desirable.^{1,19} Based on thermal stability, the backbone possesses high resistance to oxidative decomposition at a temperature around 300 °C, and above this temperature, the polymer usually depolymerizes to cyclic oligomers.^{26,28} There is a lone pair of electrons on the nitrogen with which the backbone uses to bind transition metal complexes or protons, and this lays the basis for the formation of catalytic or chemotherapeutic polymers (such as *cis*-platinum), and polymer-metal complexes (with catalytic or electronic properties).⁵ Finally, due to the P–N backbone, higher refractive index is observed in polyphosphazene polymers than it is in most other polymers. This feature is vital in the electro-optical devices.³⁵

B. Side group effect

The properties of the polyphosphazene polymers are controlled by the side groups linked to the backbone through the

phosphorous atoms.^{26,28,54–56} The glass transition temperature (T_g) is dependent upon the side groups used during macromolecular substitution as they can adjust the barrier to torsion of the backbone, thus changing T_g over a wide range of temperatures.^{1,55} Polyphosphazenes with small-sized side groups (such as methoxy or ethoxy) or highly flexible side groups (such as methoxyethoxyethoxy) exhibit elastomeric properties and have low T_g .^{1,21,37,55} This is attributable to the minimal restriction or barrier imposed by these groups on the mobility of the backbone.¹ On the contrary, large side groups (such as phenylphenoxy, ferrocenyl, carboranyl, biphenyleneoxy, naphthaleneoxy) or side groups with several hydrogen sites (such as peptide ester, phenylamino) yield brittle polyphosphazenes with high glass transition temperatures.^{20,35} The hydrolytic stability of the polymers also depends on the side groups as certain organic side groups will leave the backbone vulnerable to a hydrolytic attack, which could ultimately lead to skeletal bond cleavage.^{1,57} For example, hydrolytically sensitive groups such as amino acid esters linked via the nitrogen terminus to phosphorus, peptides, glucosyl, or glyceryl would sensitize the backbone to hydrolysis.^{54–56} This has been used as a deliberate design strategy to cause a hydrolytic breakdown of the polyphosphazenes to phosphate, ammonia, and corresponding side groups.^{1,14,28} This is an approach of interest for biomedical applications such as regenerative engineering and drug delivery. Meanwhile, the incorporation of hydrophobic groups such as aryloxy into the backbone would hamper hydrolysis.^{1,25} Therefore, degradation rates of a degradable polyphosphazene-based material can be modulated by the introduction of different ratios of hydrophilic (hydrolysis-promoter) and hydrophobic (hydrolysis-inhibitor) groups to the skeletal backbone.^{19,25}

C. Architecture

Polymer architecture plays a significant role in defining the chemical and physical properties of polyphosphazene polymers.^{28,43} Polyphosphazenes exist in many different architectural arrangements such as linear polymers, lightly branched polymers, highly branched polymers, star or dendritic structures, comb polymers, and a spectrum of block-copolymers and macromolecules with six-membered rings.^{17,20,28} These architecturally different polyphosphazene-based polymers show different and unique properties.^{26,43} For example, a linear polyphosphazene with similar side groups and the same molecular weight as a star-structured polyphosphazene polymer would exhibit different properties.²⁸ This difference in properties is due to their architectural difference. The same phenomenon applies to a comparison of a linearly arranged polyphosphazene with trifluoroethoxy side groups and a compact tristar structured polyphosphazene with the same side groups.^{20,28,33} There is a remarkable difference in the morphology and viscosity of the two polymers as the one with linear structure exhibits a semicrystalline morphology and high viscosity in solution.^{20,28} In contrast, the tristar-structured polyphosphazene depicts low solution viscosity and amorphous morphology.^{15,20,28} For the block-copolymer polyphosphazene structures, the properties are complementary to the attributes of the two constituent homopolymers involved.¹⁷ This offers a broader possibility for the design of polymer systems that take full advantage of the

benefits of each component, while minimizing the demerits of both polymers.

IV. BIOMEDICAL APPLICATIONS

A. Rationales for biomedical interest

A significant number of synthetic polymers are hydrolytically stable and often remain inert throughout their product life cycle.^{4,20,58} Nevertheless, polymers that are hydrolytically sensitive and tend to dissociate upon the exposure to aqueous media can be of interest to biomedical applications such as matrix for regenerative engineering and controlled drug release.^{1,3,7,29,59} A distinguished group of polyphosphazenes, especially those with amino acid and dipeptide-based substituents, have shown tremendous promise in biomedicine due to their degradability and biocompatibility.^{1,5,31,57,60} Thus, polyphosphazene systems, including blends and composites have been extensively investigated by several research groups for their suitability, which is often based on the test for biocompatibility, toxicity of degradation products, and drug/enzyme immobilization capabilities.^{15,52,61} The intrinsic design flexibility, property tunability, and functional diversity provided by macromolecular nucleophilic substitution with different biologically relevant substituents are essential to this field.^{1,12,19,29,45,48} The dynamism with which the degradation rates can be efficiently regulated by the side group chemistry is critical to the development of biomaterials that serve not only as biomimetic scaffolds for tissue regeneration but as a drug carrier for extended-release of bioactive molecules.^{19,23,30}

B. Regenerative engineering

Even though numerous biomaterials have previously been employed in regenerative engineering, associated drawbacks for each, such as acidic degradation products and functional rigidity, have hindered their progress.^{32,62} Biomaterials are typically utilized to fabricate biomimetic scaffolds that provide a transient structural template and mediate cellular activities towards functional tissue development.^{5,22,31,62,63} The complexity and continuously changing demands of regenerative engineering have spurred the design of new materials with high capability and versatile functionality.¹⁹ Owing to the unique attributes and features discussed earlier, a variety of polyphosphazene-based biomaterials have been explored for the design of scaffolding materials for use in bone and skeletal tissue engineering.^{1,30,61} The use of degradable polyphosphazenes in regenerative engineering was first implemented by the research group of Laurencin and has recorded huge research success since then.^{1,37} Laurencin and collaborators have comprehensively investigated these materials and their interactions with a variety of osteoblast-like stem cells to determine their suitability for bone grafts and implants.¹ One of the initial studies involved the development of 3D and 2D matrices of amino acid-based polyphosphazene, on which osteoblast cells were seeded.⁶⁴ It was observed that the 3D construct presented pores that were similar, in shape and size, to that of natural bone. It was also noted that the 3D polyphosphazene scaffolds exhibited relatively higher cell attachment and proliferation than the 2D scaffolds did. Generally, this study

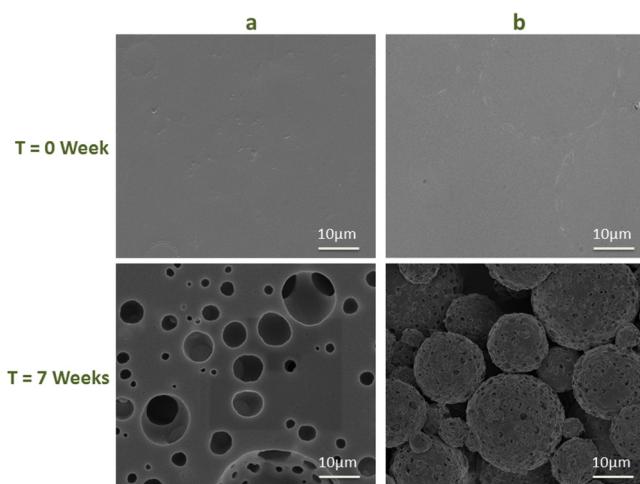


FIG. 3. SEM images showing the time-dependent changes in the morphology of dipeptide containing polyphosphazene and PLGA blends upon degradation. (a) Porosity is presented by the formation of interconnected hollow holes. (b) Porosity is presented by the assemblage of microspheres. Reprinted with permission from Ogueri *et al.* ACS Appl. Polym. Mater. **2**, 3 (2020). Copyright 2020 American Chemical Society.

signified that polyphosphazene would be appropriate for bone regenerative engineering.

Similarly, another study by Laurencin *et al.*⁵⁰ examined the suitability of polyphosphazenes for the repair of bone tissues using polyphosphazene-hydroxyapatite composites. There were favorable interactions between the composites and MC3T3-E1 osteoblast-like cells as the cells showed well-spread attachment and robust proliferation on the composite materials as compared to polystyrene-coated culture plates (TCPS). The composite systems showed enhanced mechanical properties that were appropriate for the regeneration of bone tissues, and the physical properties were maintained throughout the degradation of the materials. Once again, this study was able to illustrate the versatility of polyphosphazenes as biomaterials for regenerative engineering applications. Based on the successes

of these early studies, recent investigations have reported the design of cosubstituted polyphosphazenes (composed of two or more side groups) for use as biomaterials for bone regenerative engineering.^{12,13} In one study, Ogueri *et al.*²² demonstrated the possibility of incorporating amino acid ester/phenylphenol and dipeptide ester into polyphosphazenes to improve their suitability as biomaterials for bone regenerative engineering. The dipeptide-based cosubstituted polyphosphazenes showed a wide range of degradation rates and physicochemical properties as the ratios of the side groups were altered. This trait suggests the materials' potential utility in a variety of regenerative engineering applications as the materials can be tailored to meet specific regenerative needs. In a further study, they exploited the high numbers of hydrogen bonding sites on the dipeptide side group to create a miscible blend with PLGA.^{23,65,66} The polyphosphazene-PLGA blends showed a unique erosion mechanism that presented a porous structure with interconnectivity upon degradation (Fig. 3). This intrinsic porosity encourages cell infiltration (Fig. 4), vascularization, and in-growth of tissues for enhanced native tissue-material integration.²³ The polyphosphazene-based nanofiber technology has also been investigated for its suitability in regenerative engineering.^{30,67} For example, Deng *et al.*⁵¹ designed nanofibers based on PLGA, glycylglycine ethyl ester-phenyl phenoxy cosubstituted phosphazene, and a blend of the two polymers through electrospinning techniques. The nanofiber mats possessed fiber diameters between 50 and 500 nm, which were similar to the nano-scale of the natural extracellular matrix in native tissues. The biomimetic matrices promoted the infiltration of cells (by allowing the migration of cells from the outer blend layers into the interlayer space) and the deposition of extracellular matrix by the osteoblast cells as expressed by the phenotype marker. Holistically, this study proves that polyphosphazenes have promising prospects as biomaterials for regenerative engineering.

C. Therapeutic drug delivery

A variety of polyphosphazene, including linear polyphosphazenes and cyclo-matrix polyphosphazenes have been studied as delivery vehicles for drugs, genes, and proteins.^{45,49,52} One of the critical aspects of advanced biomaterials for tissue regeneration is the ability of the matrix to support cell growth by controlling the

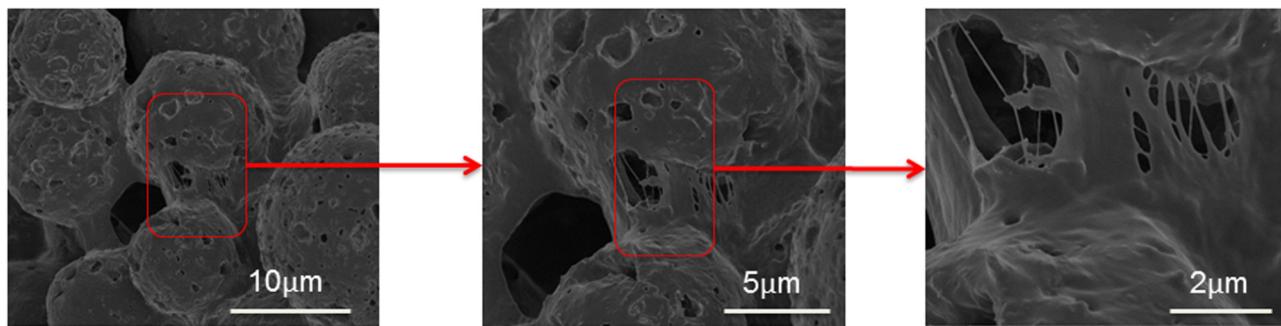


FIG. 4. SEM cell morphology showing the cell-infiltration within pores of polyphosphazene-PLGA blends. Reprinted with permission from Ogueri *et al.* ACS Appl. Polym. Mater. **2**, 3 (2020). Copyright 2020 American Chemical Society.

release of growth factors and/or drugs.⁶⁸ The drug molecules can either be covalently bonded to or encapsulated into the degradable polyphosphazenes, and the hydrolysis of the polyphosphazene backbone could result in the release of the drug molecules.^{2,52} For matrix encapsulation, early studies entailed the release of drug molecules from matrices based on the amino acid ester and/or imidazole containing polyphosphazenes.⁶⁹ Laurencin *et al.*⁷⁰ demonstrated that the adjustment of the composition of the hydrolytic sensitive side groups such as imidazole units in a cosubstituted poly [(imidazolyl)(methylphenoxy)phosphazene] could be utilized to deliver model drugs such as progesterone and serum albumin at a constant rate in *in vitro* and *in vivo* settings. In a similar study, they examined the intra-articular administration of an anti-inflammatory drug, colchicine, using polyphosphazene polymers with imidazolyl (I-PPHOS) or ethyl glycinato (EG-PPHOS) side-chain substituents.⁷¹ The release of colchicine was 20% for I-PPHOS and 60% for EG-PPHOS over the 21-day study, and these results were similar to naproxen sustained release systems.⁷² The use of degradable polyphosphazenes for protein release has also been investigated.^{52,73} For instance, degradable poly [(*p*-methylphenoxy) (ethylglycinato) phosphazene] matrices loaded with insulin have shown a great promise for its suitability for drug delivery.⁷⁴ The release of insulin was dependent on pH as lower pH values resulted in an increased release rate. It was intriguing to know that the protein loading content influenced the degradation rate, and this is attributed to the increased hydrophilicity of the matrix with higher quantities of protein. Another study by Caliceti *et al.* reported the loading of insulin with microspheres based on phenylalanine ethyl ester and imidazole substituents at a molar ratio of 80/20.⁴⁶ The study demonstrated that subcutaneous administration of the system to diabetic mice instigated a drop in the glucose levels, which was maintained for 100 h. The decrease in the glucose level induced the production of insulin antibody, which increased progressively for 8 weeks.⁴⁶

For anticancer drug conjugates, degradable polyphosphazenes have been conjugated with drug molecules to obtain implantable matrix devices and/or injectable microspheres.² The macromolecular substitution of polydichlorophosphazene enables the direct linkage of drugs and/or functional groups for drug loading to the polymer skeletal backbone.¹² This is a preferred method because macromolecular carriers whose drug molecules are covalently bound to them are more effective transporters than systems (such as micelle-based systems), whose drug molecules are allied to them via secondary interaction.^{75,76} The latter case often results in significant leakage of the drug molecules. Additionally, the variability of polydichlorophosphazene provides the design flexibility with which tumor-specific targeting ligands can be incorporated together with the drug molecules onto the polyphosphazene backbone to guide the drug to the target site.^{28,37,77} Also, biocompatible plasma-soluble groups such as polyethylene glycol (PEG) can be added to improve solubility and to confer the "stealth effect," which helps the drug molecules to evade the immune system by reducing protein adsorption.^{78–80} Owing to the fact that polydispersity has a bearing influence on the biodistribution of the drug molecules, polyphosphazenes with low polydispersities and controlled molecular weights, synthesized through the living cationic polymerization technique have been employed for drug delivery.⁴² An acid-sensitive hydrazide unit was used to link the anticancer drugs

epirubicin and doxorubicin (DOX) to the polymer backbone, and the unsubstituted chlorine atoms were completely displaced with polyalkylene oxide chains.⁴² Interestingly, the carrier exhibited a burst release of the drug molecules, which depended more on the pH than the erosion of the backbone. Similarly, polyphosphazene with narrow molecular weight distribution synthesized by living polymerization routes with photoactive drug hypericin covalently bound to its backbone has also been investigated for drug delivery.¹¹ It was observed that the polymers maintained phototoxicity of the free drug while improving their solubility; thus, this represents a good avenue for polymer assisted delivery for photodynamic therapy.

As mentioned earlier, the small molecule model reaction is often used to ascertain the feasibility of the macromolecular substitution of PDCP with organic groups.^{19,22} It is evident that any conjugation or substitution which applies to linear polyphosphazenes will also apply to the HCCTP monomers. For this reason, there have been several attempts to prepare cyclotriphosphazene-based conjugates for drug delivery.⁸¹ For instance, Jadhav *et al.*⁴⁷ designed a micelle-encapsulated platinum (II) anticancer agent in which the HCCPT ring was substituted with hydrophobic oligopeptides and methoxy poly(ethylene glycol) (MPEG) chains at various ratios. The amphiphilic oligomers obtained were able to self-assemble into stable micelles when placed in aqueous media and was used to encapsulate a hydrophobic and water-insoluble platinum (II) compound, *cis*-(cha)₂Pt(NO₃)₂ drug molecules (Fig. 5). The micelle-encapsulated drug compounds exhibited extended blood circulation times in rats, and the biodistribution study of the system showed tumor selectivity and high cytotoxicity toward tumor cells as compared to the free drug. Besides, Jun *et al.*⁸² also demonstrated that micelle forming Pt anticancer drugs can be covalently bonded to the ring to give an amphiphilic cyclotriphosphazene-platinum (II) conjugates. Similar chemistry based on the self-assembly of cyclotriphosphazene compounds was reported, where cyclotriphosphazene derivatives were substituted with tryptophan ethyl ester units to obtain hexa-[*p*-(carbonyl tryptophan ethyl ester) phenoxy] cyclotriphosphazene (HEPCP). The HEPCP self-assembled into nanoparticles with excellent thermal stability and strong fluorescent emission.⁸³ Thermosensitive polyphosphazenes have also been explored to study the release of drug molecules. Zhang *et al.*^{84,85} demonstrated that poly

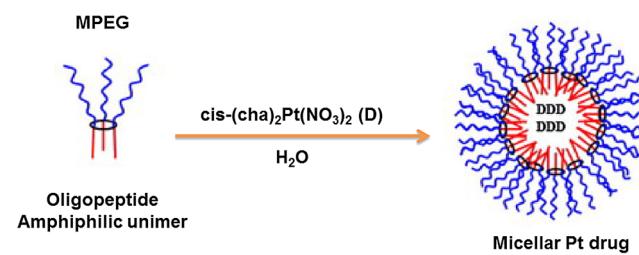


FIG. 5. Schematics showing the formation of cyclotriphosphazene amphiphilic micelles and the encapsulation of drug molecules (DDD). Oligopeptide amphiphilic unimer self-assemble into stable micelles when placed in aqueous media. Reprinted with permission from Jadhav *et al.* *J. Control. Release* **147**, 1 (2010). Copyright 2010 Elsevier.

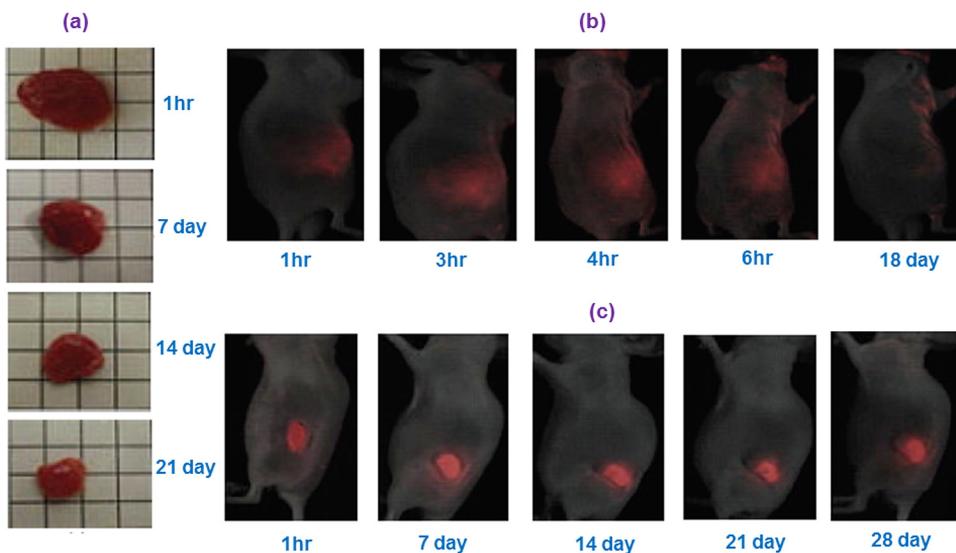


FIG. 6. Images showing the *in vivo* degradation and localization of polyphosphazene-DOX conjugate. (a) Gradual mass loss with time due to the biodegradation of intratumorally injected polyphosphazene-DOX conjugate. (b) Time-dependent fluorescent emission of intratumorally injected DOX solution showing the delocalization of DOX molecules. (c) Time-dependent fluorescent emission of poly(organophosphazene)-DOX conjugate hydrogel showing the highly localized DOX molecules, whose release is controlled by the hydrogel. Reprinted with permission from Chun *et al.* *Biomaterials* **30**, 27 (2009). Copyright 2009 Elsevier.

(*N*-isopropyl acrylamide) (PNIPAm)-containing polyphosphazene could be triggered by temperature change to self-aggregate into micellar structures with lower critical solution temperature around 30 °C. With the same PNIPAm-grafted polyphosphazene, it has also been illustrated that hydrophobic drug ibuprofen can be solubilized to polymeric aggregates.⁸⁶ The nanospheres obtained from the aggregates must release the drug molecules via diffusion from the polymer matrix as the degradation rates of the polyphosphazenes were slow and did not tally with the time frame of the release experiments. The ability to impose temperature-responsive sol-gel transition behaviors on polyphosphazene-based polymers is an excellent avenue for making injectable hydrogels for drug delivery.¹⁶ Hydrophilic PEG chains and hydrophobic isoleucine ethyl groups have been utilized in a macromolecular cosubstitution to yield a thermosensitive polyphosphazenes, which were employed for the sustained delivery of model drug 5-fluorouracil and the anticancer drug DOX.^{87–89} Hydrolytically active groups such as glycyl lactate ethyl esters can be incorporated to increase the rate at which the polymers degrade. At a physiological temperature of 37 °C, the polyphosphazene-DOX solution showed a transition from an aqueous form to a hydrogel [Fig. 6(a)].⁸⁹ Cho *et al.*⁹⁰ showed that similar polymers could augment the bioavailability of hydrophobic silibinin, which was sustainably released for improved antitumor effects. The structural diversity and multiplicity of polyphosphazene backbone have been capitalized on to link several thiol groups to the polymer. The thiol units provided chemical crosslinking sites for further improvement of the mechanical strength of the gel.⁹¹

Another exciting area for which polyphosphazene-based drug devices can be of enormous use is precision medicine. Precision or personalized medicine is of great interest in the modern medical diagnostics and treatment of many diseases as it allows the customization of healthcare products to an individual patient.³⁸ The design flexibility, tunable degradation rates, mechanical strengths, and stimuli-responsive properties of polyphosphazenes are an asset and could be utilized to fabricate precision biomaterial-based devices that

can target and treat various diseases.^{89,92} This ensures the administration of appropriate and optimal therapies based on individual patient complexity and personal characteristics such as gender, age, and ancestry.⁹² Therefore, polyphosphazene polymers will continue to rise to the occasion and inspire innovative solutions in drug delivery applications.

Cyclo-PPHOS materials have been reported to exhibit strong fluorescence when incorporated with a fluorescent compound. Meng *et al.*⁴⁸ showed that poly(cyclotriphosphazene-*co*-dibromofluorescein) (PCTPDBF) has the capability to emit colored fluorescence at any concentration. The PCTPDBF nanoparticles were seeded with HeLa and H9C2 cells to demonstrate the cell imaging application (Fig. 7). The cell-loaded PCTPDBF displayed excellent biocompatibility and high resistance to photobleaching as the PCTPDBF nanoparticles emitted green fluorescence under irradiation of 450 nm laser [Figs. 7(b) and 7(e)]. While the nuclei of cells exhibited bright blue fluorescence under irradiation of 360 nm laser when stained with 4',6-diamidino-2-phenylindole dihydrochloride [Figs. 7(a) and 7(d)]. The combination of the blue and green pictures yielded perfect confocal laser scanning microscopic cell images [Figs. 7(c) and 7(f)]. In a similar study, Wang *et al.*⁴⁹ integrated dual functions of imaging and drug delivery into a single device for simultaneous diagnosis and therapy of cancerous cells. The device was based on poly(cyclotriphosphazene-*co*-dichlorofluorescein) (PCTPDF) nanoparticles (designed via the polycondensation of HCCTP and FD) and linked with different drug molecules (folic acid, PEG-NH₂, and methylene blue). The PCTPDF multifunctional platform was compatible with seeded cells, and also, when injected into a genetically engineered mouse, it showed excellent *in vivo* imaging capability. Similarly, another study further demonstrated the *in vivo* antitumor activities and the fluorescent ability of a locally injected poly(organophosphazene)-DOX conjugate.⁸⁹ After the local injection at the tumor site, there was more effective inhibition of the growth of the tumor cells by the conjugate than by the DOX alone [Figs. 6(b) and 6(c)]. In addition to tumor-growth inhibition, the conjugate

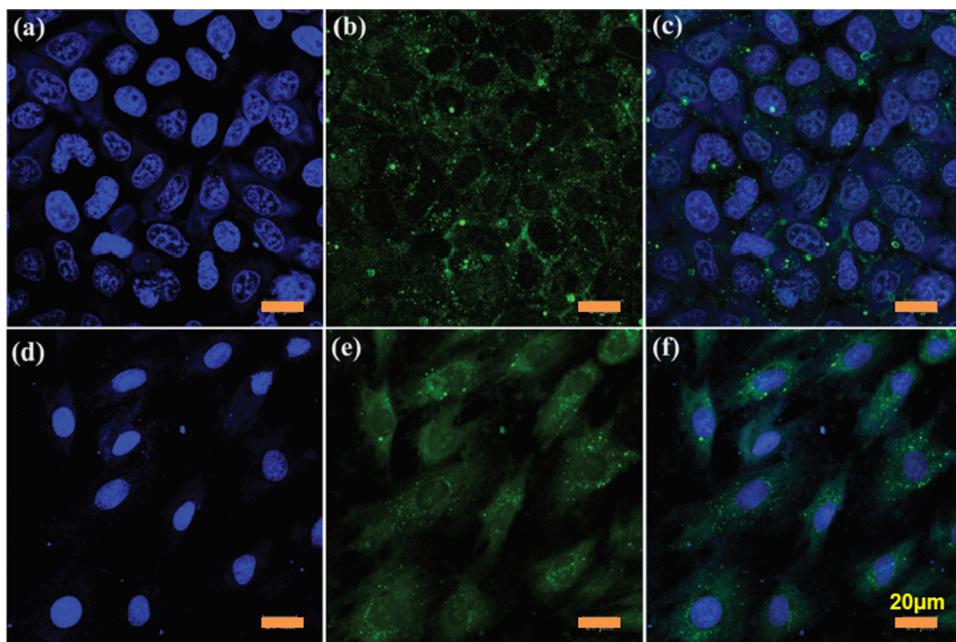


FIG. 7. Fluorescence images of (a)–(c) HeLa cells and (d)–(f) H9C2 cells seeded in PCTPDBF. Both cells emitted bright blue fluorescence at 360 nm and green fluorescence at 450 nm. Reprinted with permission from Meng et al. *Polym. Chem.* **6**, 16 (2015). Copyright 2010 Royal Society of Chemistry.

recorded less toxicity and delayed release of its antitumor DOX molecules. This suggests that there was a controlled release of DOX over an extended period, which effectively accumulated locally in the target tumor cells. Once more, poly (organophosphazene)-based conjugates have proved its promising status as a dual-functional diagnostic and therapeutic tool.

V. CONCLUSION

As interest continues to grow for the use of biodegradable polymers in biomedicine, there is a pressing demand for new and innovative polymeric materials with appropriate properties for each specific medical protocol. Polyphosphazene-based biomaterials are a unique class of polymers that offer exceptional benefits rarely found in conventional polymers such as PLGA and PCL. Although their utilization in the biomedical field has not been fully actualized, recent research progress offers a ray of hope for the future as the polyphosphazene polymers have been reported to show promising characteristics in their applicability for tissue regeneration and drug delivery. An integrative approach to moving away from the classical “ring-opening polymerization” to facile “one-stage polymerization” method by precipitation polycondensation of HCCTP would not only simplify the synthesis of polyphosphazene-based polymers but would facilitate the scale-up and commercialization of this interesting class of polymers. This review paper gave an overview of the synthetic methods, the structure-property relationships, and the underlying principles and rationales for their use in regenerative engineering and therapeutic drug delivery system.

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